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LDRD Final Report on Light-Powered Nanovehicles

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Huifang Xu, Yingbing Jiang, Frank van Swol, Yan Qiu and James E. Miller

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Abstract

We have investigated the possibility of constructing nanoscale metallic vehicles powered by biological motors or flagella that are activated and powered by visible light. The vehicle's body is to be composed of the surfactant bilayer of a liposome coated with metallic nanoparticles or nanosheets grown together into a porous single crystal. The diameter of the rigid metal vesicles is from about 50 nm to microns. Illumination with visible light activates a photosynthetic system in the bilayer that can generate a pH gradient across the liposomal membrane. The proton gradient can fuel a molecular motor that is incorporated into the membrane. Some molecular motors require ATP to fuel active transport. The protein ATP synthase, when embedded in the membrane, will use the pH gradient across the membrane to produce ATP from ADP and inorganic phosphate. The nanoscale vehicle is thus composed of both natural biological components (ATPase, flagellum; actin-myosin, kinesin-microtubules) and biomimetic components (metal vehicle casing, photosynthetic membrane) as functional units. Only light and

storable ADP, phosphate, water, and weak electron donor are required fuel components. These nano-vehicles are being constructed by self-assembly and photocatalytic and autocatalytic reactions. The nano-vehicles can potentially respond to chemical gradients and other factors such as light intensity and field gradients, in a manner similar to the way that magnetic bacteria navigate. The delivery package might include decision-making and guidance components, drugs or other biological and chemical agents, explosives, catalytic reactors, and structural materials.

We expected in one year to be able only to assess the problems and major issues at each stage of construction of the vehicle and the likely success of fabricating viable nanovehicles with our biomimetic photocatalytic approach. Surprisingly, we have been able to demonstrate that metallized photosynthetic liposomes can indeed be made. We have completed the synthesis of metallized liposomes with photosynthetic function included and studied these structures by electron microscopy. Both platinum and palladium nanosheeting have been used to coat the micelles. The stability of the vehicles to mechanical stress and the solution environment is enhanced by the single-crystalline platinum or palladium coating on the vesicle. With analogous platinized micelles, it is possible to dry the vehicles and re-suspend them with full functionality. However, with the liposomes drying on a TEM grid may cause the platinized liposomes to collapse, although probably stay viable in solution. It remains to be shown whether a proton motive force across the metallized bilayer membrane can be generated and whether we will also be able to incorporate various functional capabilities including ATP synthesis and functional molecular motors. Future tasks to complete the nanovehicles would be the incorporation of ATP synthase into metallized liposomes and the incorporation of a molecular motor into metallized liposomes.

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Introduction

Our original idea of how a nanovehicle and its basic power system would be fabricated entirely by self-assembly and self-compartmentalization processes is illustrated in Fig 1. These nanoscale metallic vehicles would be powered by biological motors that could be activated and driven by visible light. We expected that the vehicle's body would be composed of the surfactant bilayer of a liposome, which had been coated with metallic nanoparticles that are grown together into a porous single crystal. This metallic shell could be synthesized on the inside (as shown) or outside

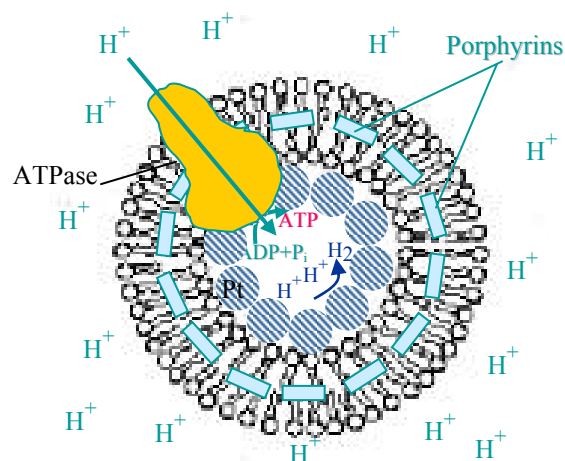


Fig 1. The nanovehicle and its power source. Light induces H₂ evolution and a pH increase within the metallized liposome. The pH gradient across the liposomal bilayer is used by ATP synthase, a molecular motor itself, to produce ATP. The ATP produced can power other molecular motors such as myosin and kinesin.

surface of the surfactant bilayer of a liposome by a photocatalytic approach being developed at Sandia. The power components of this nanovehicle, when exposed to light, generate a proton gradient across the bilayer membrane which could be used to drive the rotary motor of a flagellum to move the vehicle (see below). However, incorporating the flagellum or other molecular propulsion systems into liposomes is a challenging problem. The flagellum would have to be properly anchored into the membrane while allowing the flagellum to rotate, and it is not yet possible to reconstitute functioning flagella in this way, though a several groups are working on this problem. Instead, linear motion could be achieved using kinesin motors to carry the metallized liposome by walking on microtubules, a technology being developed by George Bachand at Sandia. The nanoscale vehicles envisioned are thus composed of both natural biological components (ATPase, molecular motors) and biomimetic components (vehicle housing, artificial photosynthetic membrane) as functional units. Only light and storable ADP, P_i, water, and a weak electron donor such as a tertiary amine are required to fuel both motor and delivery-package components. These nanovehicles will be constructed entirely by self-assembly and photocatalytic processes.

The ultimate goal is to develop a nanoscale vehicle that can move about to deliver a payload to a specific site. The payload might be (1) the catalytic capability to produce a specific chemical or biochemical, (2) the ability to sense chemical or biological agents, (3) the ability to

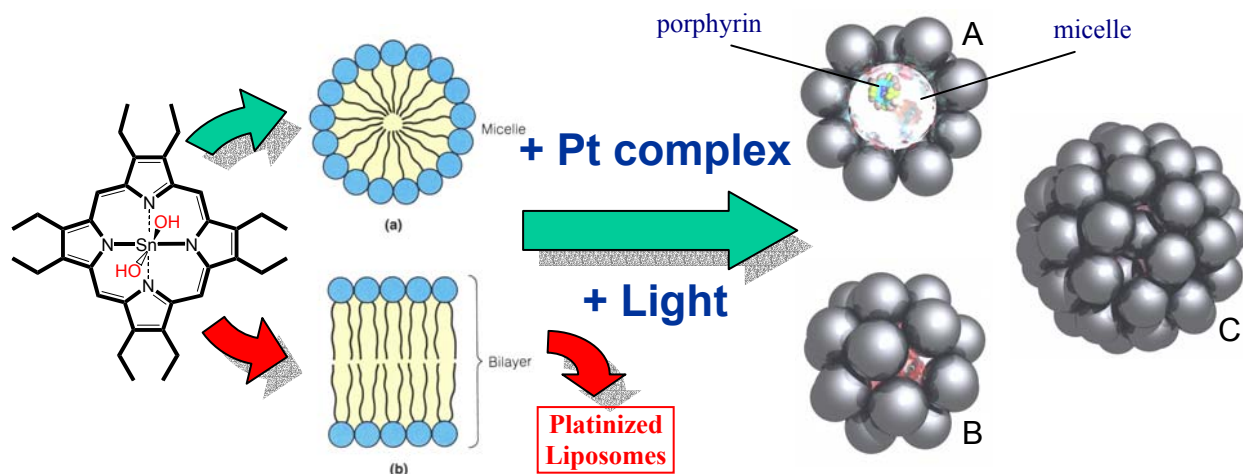


Fig 2. Synthesis of platinized micelles and liposomes. For the micelles, Sn octaethylporphyrin is added to a solution of sodium dodecylsulfate (SDS) above the critical micellar concentration (green arrows). This SnOEP solution is added to a solution of Pt complex and ascorbic acid and, under intense illumination with visible light, a Pt nanodendrite is grown on the micelle. (A) a partially coated micelle, (B) a fully coated micelle, and (C) a larger nanodendrite on the micelle. TEM images show that each nanodendrite is a single crystal. Taking the bottom route (red arrows) leads to metallized liposomes as illustrated in Fig 1, the main goal of this work.

spatially sort and organize molecules, (4) explosives, (5) nanoscale chemical reactors, and (6) construction materials. If active payloads were constructed properly, different light-driven components within the vehicle might be turned on and off by using different colors of light, giving one the ability to move the nanovehicles and switch on their payloads independently.

Previously, we had already demonstrated the construction of similar nanostructures. In this case, the metal was crystalline platinum and the surfactant substrate was sodium dodecylsulfate (SDS) micelles instead of liposomes. Micelles are small surfactant assemblies that are essentially composed of only a single layer of surfactant, as illustrated in Fig 2. We had shown that it was possible to photocatalytically grow nanodendrites of Pt on the surface of micelles of SDS, and Fig 2 illustrates this synthetic process. We have now extended this synthesis to the lipid bilayer surfaces of liposomes to create our nanovehicles. Unlike micelles, liposomes are spheroidal bilayer structures that have an interior and an exterior surface and they enclose solvent in the interior space. Liposomes of between 100 nm and a few microns are easily produced by extrusion of a surfactant solution through pores of a particular size.

For the platinized surfactant assemblies, first a water-insoluble porphyrin is dissolved in the surfactant solution, which is then diluted and sonicated to form an aqueous solution containing the photocatalyst molecules. Next, a Pt(II) salt solution, containing mostly $\text{PtCl}_2(\text{H}_2\text{O})_2$, and an electron donor (ascorbic acid) are added and the solution containing the desired surfactant assembly is illuminated with light from a tungsten lamp. In this system, photoactivated electron

transfer from the porphyrin to reduce Pt(II) to the zero-valent metal occurs at the surfactant surface. Within 5 minutes, the metal ions are reduced giving a platinized surfactant assembly containing the still active photocatalyst inside as indicated for micelles in Fig 2.

Accomplishments

We have fully studied the platinum-reduction reaction just described and found that under certain conditions the reaction also involves autocatalytic reduction of Pt(II) at the surface of growing metallic platinum nanodendrites. That is, porphyrin-mediated photocatalytic reduction of Pt(II) grows platinum nanoparticles, which can then grow rapidly by autocatalytic oxidation of electron donors like ascorbic acid to produce 2- or 3-dimension nanodendrites.

TEM images of the amazing 3-dimensional nanodendrites grown on micelles are shown in Fig 3. The TEM studies show that the nanodendrite grown on each micelle is a single crystal. The crystallinity of the entire nanodendrite surrounding the micelle makes the nanoassembly a robust structure that survives for months in solution and even survives drying and re-suspension by sonication.

We have generalized the synthesis of the micellar nanoassemblies to make platinized liposomal nanoassemblies like that illustrated in Fig 4 and similar to that proposed in Fig 1. For the platinized liposomes, the photocatalytic porphyrin is located in the lipid bilayer. After the Pt complex is depleted from solution, the photocatalytic tin porphyrin remains active, *i.e.*, light is still able to drive the porphyrin-based photocatalytic reaction in the bilayer and thus electrons that would go into reducing

Pt ions the go to the platinum metal, which is a well-known catalyst for reducing water to H₂. We have measured the efficiency of this reaction for the platinized micelles of Fig 3, and fully expect the reaction to be even more efficient for the platinized

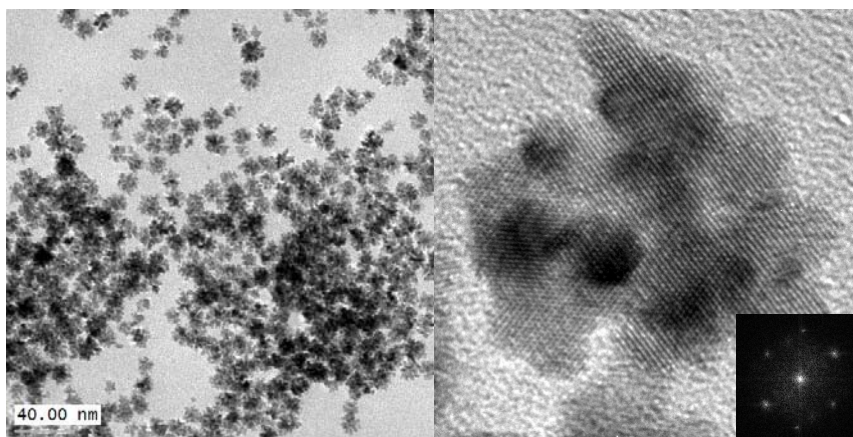


Fig 3. Platinized micelles of controlled size produced by the photocatalytic generation of initial growth centers followed by autocatalytic growth into nanodendrites. The nanodendrites are crystalline as shown by the atomic fringing evident in the high magnification TEM image on the right.

liposomes. For the platinized liposomes, H_2 evolution reaction takes up protons from water, raising the pH and generating a pH gradient across the liposomal membrane. Although the platinized micelle nanodendrites evolve hydrogen, they could not serve as useful nanovehicles because they have only rudimentary compartmentalization of function.

Further, the development of the light-harvesting arrays presents a major problem for the micellar nanoassemblies because their small size makes the incorporation of a sizable light-harvesting array virtually

impossible. In contrast, the the platinized liposomal nanostructures like that illustrated in Fig. 2 could allow a light-harvesting array to be included because of the large area of the liposomal membrane compared to the Pt coating. Another advantage is that water-soluble and water-insoluble porphyrins tend to self-organize both in the aqueous interior of the liposomes and within the hydrophobic environment of the liposomal membranes; the latter arrangement of the light receptors is preferable for energy transport. For these reasons, we concentrated our effort on producing free-standing platinized liposomes for nanovehicle bodies.

We were encouraged when it was found that using large liposomes as a template produced dendritic disk-like sheets (nano-caps) or solid foam-like nanomaterials (also see Fig 6). TEM images of the nanocaps produced are shown in Fig 4. This suggested that we might be able to coat the liposomes with the 2-nm thick platinum sheeting rather than the 3-dimensional nanodendrites shown in Fig 3. Because of the dendritic nature of these 2-dimensional dendrites, the platinized liposomes will retain the needed porosity on the scale of small molecules while providing a thin platinum jacket around the liposome.

Further indications that individual liposomes might be coated with the Pt nanosheet came from TEM and SEM of the nanofoams shown in Fig 5. The particular type of morphology obtained for the foams depends on light exposure, solution conditions, and size of the liposomes.

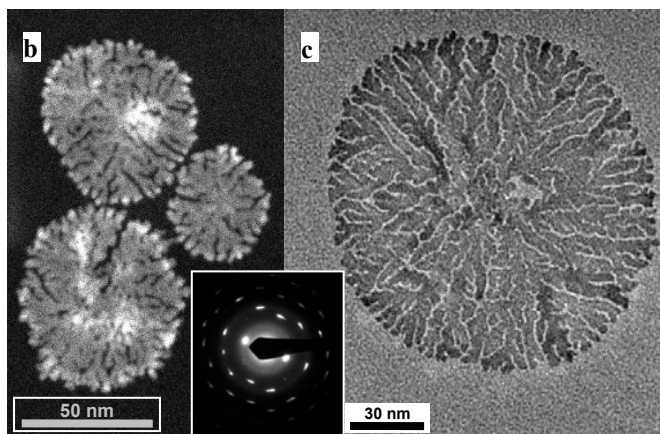


Fig. 4. Dendritic platinum nanocaps templated by liposomes. (a) HAADF scanning TEM image of three platinum nanocaps grown on 160-nm diameter DSPC liposomes. (b) TEM image of a large dendritic Pt nanocap made using DSPC liposomes and (Inset) its electron diffraction pattern. The surfactant assemblies cannot be seen in the TEM images because of the lack of contrast of carbon with the much denser Pt and the interference from the carbon film of the TEM grid.

Control over the morphology is provided by the diameter of the templating unilamellar DSPC/cholesterol

liposomes. The large cavities in the foams are determined by the size of the liposomes; when 65-nm liposomes are used in the reaction, the cavities in the foams have this average size, and similarly when 120-nm or 165-nm liposomes are used instead, the cavities in the nanofoams reflect the

larger liposomal size. These cavities are particularly evident in the SEM images of Fig 5. These results suggested that with not much more control over the structure obtained by varying the liposome size, photo-catalyst concentration, light exposure, and solution conditions, we might be able to produce free-standing platinized liposomes.

For the Pt foams, we have indeed achieved additional control mainly by using the porphyrin photocatalyst incorporated into the liposomes. Exposure of the reaction solution to incandescent light produces smaller balls of the foam and a more uniform ball size distribution (Fig 5). Varying the light exposure and porphyrin loading at constant platinum-salt concentration determines the size of the foam balls and even gives continuous foam phases under certain conditions. This suggested that we might also vary the Pt concentration and light exposure to obtain individual liposome coated with 2-nm thick, 2-

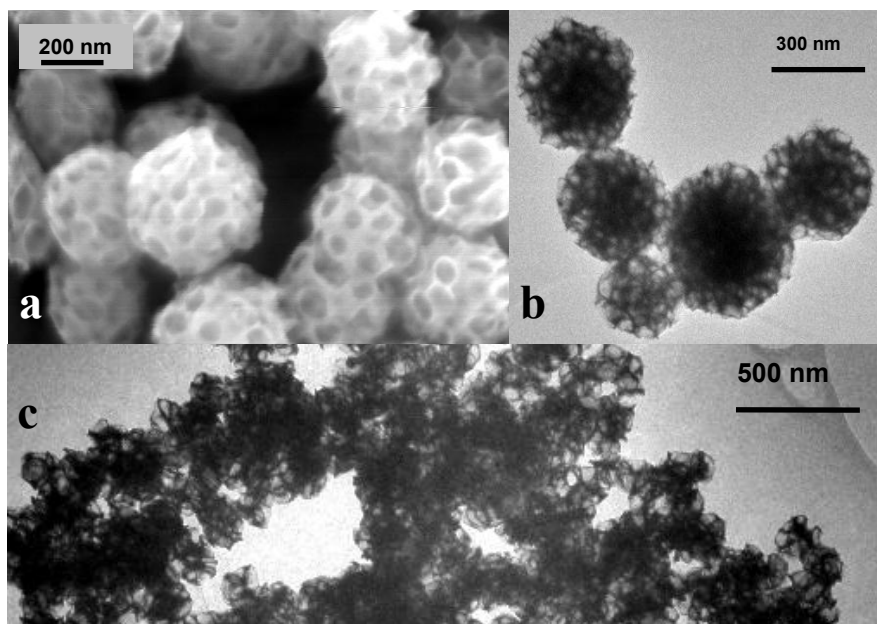


Fig 5. Foam-like balls composed of platinum nanosheets grown at the interfaces between aggregated DSPC liposomes. The balls have a uniform size because the porphyrin photocatalyst initiates growth within the liposomal aggregates, and growth occurs along the interfaces between the liposomes in a spherically symmetric manner until the Pt(II) is exhausted. (a) SEM image of the platinum foam balls. (b) TEM image of the foam balls. (c) Under certain condition the growth centers are close enough so that growth leads to a continuous phase, which retains the nanoscale pore size dictated by the liposomal template.

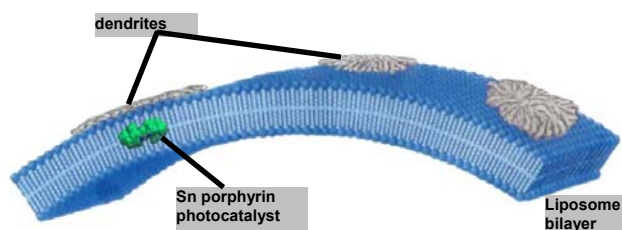


Fig 6. Dendritic platinum nanocaps templated by liposomes; illustration of the growth of Pt nanocaps on the liposomal surface.

dimensional nanodendrites. An early stage in this process is illustrated in Fig 6, where porphyrins in the liposomal membrane initiate the growth of nanodendrites that grow together to coat the entire surface.

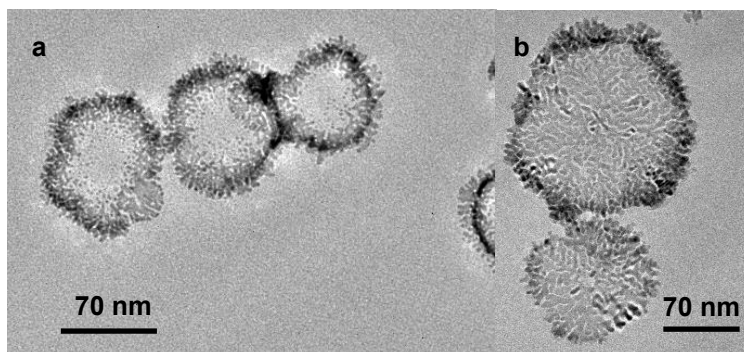


Fig 7. (a) Platinized liposomes. (b) Liposomes coated with nanometer-thick palladium dendritic sheets.

The nanoscale porosity is particularly evident in the isolated nanosheets or nano-caps that can be grown on the liposomes under certain condition (Figs 4(a) and 4(b)). The nanocap growth process is illustrated in Fig 6. The TEM images of the nanocaps in Fig 4 show the dendritic nature of the 2-dimensional nanostructures; the platinum metal foams are also clearly made up of joined 2-dimensional dendrites. The platinum nanofoams will have potential applications in catalysis because of their high surface area, the ability to control their porosity on different length scales, and the possibility of tailoring their structural stability. Fuel cells may be one commercial area in which the platinum nanofoams may have advantages.

Most importantly for the nanovehicles, these liposomal structures coated with platinum sheeting further indicated that reaction conditions could be found that would allow individual liposomes to be fully coated with Pt metal. Indeed, varying the reaction conditions has produced nanostructures for which TEM images clearly indicate the formation of liposomes with near complete coatings of either the nanosheets or the 3-dimensional dendritic platinum. TEM images of some liposomes coated with platinum or palladium metal are shown in Fig 7. The success in producing the platinized liposomes relies on the ability to increase the concentration of the photocatalytic porphyrin within the DSPC/cholesterol surfactant bilayer. Accomplishing this proved difficult, but ended up providing the desired conditions.

Fig 8 shows the density profile of the large palladium coated liposome in Fig 7(b). For a spherical shell of thin platinum sheeting, one would

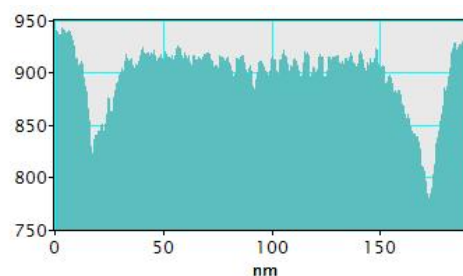


Fig 8. Density profile of the large liposome coated with nanometer-thick palladium dendritic sheets shown in Fig 7. The profile suggests that the liposome is fully coated—Pt at the perimeter is thicker than in the middle suggesting a spherical structure which may have collapsed upon drying.

expect such a profile, with much platinum at the perimeter and only thin sheets for most of the interior. The metallized liposome may have collapsed upon drying on the TEM grid.

We have also been able to metallize liposomes with what appears to be a coating of the nanodendrites of the form observed on SDS micelles. A TEM image of these metallized liposomes is shown in Fig 9. Nanovehicles based on this type of nanostructure would also be possible and may offer some advantages over those jacketed with the 2-nm thick dendritic metal sheets.

Summary and Conclusions

The work accomplished so far has clearly demonstrated all of the elements required to produce a liposome coated with Pt nanosheets—the body of our nanovehicle—with the capability of evolving molecular hydrogen as a means of producing a proton gradient across the membrane when exposed to light. It remains to be shown that these interesting nanostructures can be put to use as fully functional, light-powered vehicles that can carry useful cargos. If ATP synthase (ATPase) is embedded in the membrane, it should use the pH gradient to produce ATP from ADP and inorganic phosphate (P_i). ATPase has been reconstituted into liposomes by others; the ATP produced could fuel any number of desirable biochemical reactions including motive systems.

The most attractive motile system to attach to the metallized liposomes would be a bacterial flagellum with its motor directly incorporated into the liposomal membrane. The flagellum motor does not require ATP, but is powered directly by the proton gradient across the plasma membrane. It rotates at about 100 revolutions per second, powered by passive transport of protons back across the membrane. Unfortunately, we do not yet have the ability to reconstitute flagella into liposomal membranes, although this capability may become available in the next few years. In fact, the structural rigidity of the Pt shell around the liposome may aid in the incorporation of natural flagella.

A more viable alternative approach is to use other biological motors and processes to propel the nanovehicles using the light-generated ATP as fuel. For example, vesicle-attached kinesin-

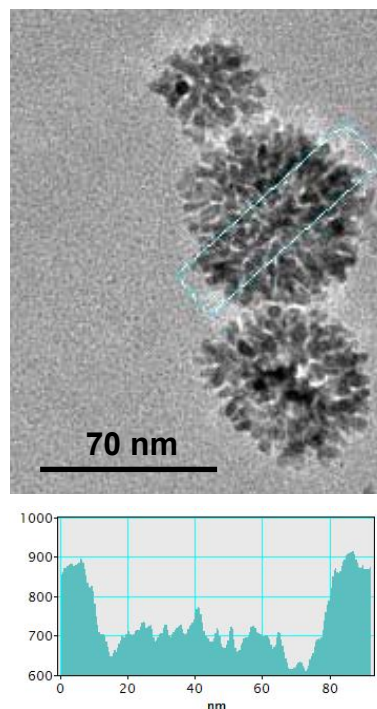


Fig 9. Liposomes coated with Pd metal dendrites similar to those coating SDS micelles and the density profile of one of the metallized liposomes.

like molecular motors might move the vesicle along a microtubule. The kinesin-microtubule system is the system by which vesicles and organelles are transported within cells. The microtubules form a railway system and kinesin connected to vesicles walks along the microtubule rails carrying the vesicles with it. A single kinesin motor can move a vesicle along a microtubule. The problems to be solved here are how to attach the kinesin motor to our liposome and how to get the ATP produced to the kinesin motor. This most likely will require the reverse configuration to that shown in Fig 1, *i.e.*, the Pt nanoparticles should be on the exterior and the ATPase should be inverted in the membrane, producing ATP outside the liposome and near the kinesin motor.

Liposomes with incorporated photosystems that pump electrons vectorially across the bilayer membrane are being developed by others using carotenoid-porphyrin-quinone triads, and liposomes with incorporated chloroplast ATPase enzymes have produced both a proton motive force and an inter-membrane potential. Our new approach uses a metallic shell to increase stability a much simpler and efficient photosynthetic system. The previous work on soft liposomes demonstrates the chemical validity of our approach, but the soft liposomes lack the additional advantages offered by our metallized liposomes. The stability of these vehicles to mechanical stresses and solution conditions seems to be enhanced by the single-crystalline metal coating on the vesicle. Evidence for this comes from the TEM studies, which show that these nanostructures survive the stresses of drying during sample preparation. The enhanced structural integrity of our metallized liposomes is expected to greatly aid in the attachment molecular motors. The lack of a known method of attachment is now the major impediment to producing mobile liposomes. One method of attachment might be to include gold nanoparticles on the outer surface and modify the motor proteins with self-assembling thiol groups.

There are substantial risks associated with such radical biomimetic technologies, not the least of which is the question of whether effective motile systems can be incorporated even with the potential advantages offered by the proposed metallized liposomal nanoassemblies. Nevertheless, an attempt to use the metallized liposomes is appropriate given that they represent a radical departure from conventional liposomes and given that our approach is entirely different and potentially more robust.

The applications of such nanoscale vehicles seem limitless provided that these nanobots can be made to move about and deliver a payload to a specific site. The delivered package might

include decision-making and guidance components, drugs and other biological/chemical agents, explosives, and structural materials. The payloads might be biomedical in nature such as delivering catalytic or enzymatic activity capable of correcting biochemical deficiencies. Both biomedical and non-biomedical application of the ability to transport various sensor capabilities at the nanoscale can be envisioned. The ability to spatially sort and organize molecules at the nanoscale could have interesting environmental and remote construction applications. Imagine mining nanovehicles that hunt and retrieve valuable resources from mineral slurries. They might transport, concentrate, and ignite explosives payloads on a nanoscale. They might also provide mobile nanoscale chemical reactors for combinatorial procedures or provide chemical power in spatially confined regions. If active payloads were constructed properly, different light-driven components within the vehicle might be turned on and off by using different colors of light, giving one the ability to move the nanovehicles and switch on their payloads independently. These nanovehicles can potentially respond to chemical gradients and other factors such as light intensity and field gradients.

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